VDS Staff: No lab report due for this one until after the Next wet lab (Characterization) - all they have to do is **Post** their Images from the lab to the **Wikispaces and fill in their lab notebook**

**They work in pairs - But they need to have their own samples for the gel in the end.**

**So each person saves Samples 2,3,4,5,6 along with their Sample 1 from last lab**

There are 2 parts to the lab

PART 1: Purification

PART2: Nanodrop to measure amount of protein (this does not have to be done at the same time – but if the Nanodrop is available, they should do it)

The first steps are incubation steps that take a while. So, – to save time - they should immediately start these and while they are waiting they can

1. Do pre lab calculations

2. Download the Journal Paper and look up the Maximum Wavelength for the last part of lab

They can work in **pairs** – but must keep **samples 2, 3, 4, 5, and 6 of their own**

**REAGENTS:**

Don’t let them use TOO much Ni-NTA resin - it is expensive.

They should MIX the bottle first – then pipette out the right amount

Benzonase and Cyanase are basically the same thing (different companies)

Don’t let them set the Cyanase out too long (must keep on ice)

**TUBES TO USE**: For the Flow Through and Wash steps: use the ‘**really cheap’**, non-sterile tubes that we used for the Beer’s law lab. Use tape to label these – since they can be washed and re-used.

However, for the Elution 1 and 2: use ‘**fancy’** **purple** screw cap tubes

**IMPORTANT CONCEPTS & SKILLS:**

* Emphasize keeping the protein and re-agents cold because the protein could denature.
* Our protein is soluble and is found in the soluble fraction after clarifying the lysate
  + (from the cytosol – not a membrane bound protein)
* The HIS tag on our protein binds to the Nickel on the beads (resin = beads = matrix)
* The HIS tag is not ‘natural’ – it was put on their by genetic modification
* What imidazole does? It competes with the Histidines of the HIS tag for the binding with Nickel. If you add enough imidazole – the protein will come off the beads.

QUESTIONS TO ASK

1. First ask – ‘What is in the tube?’ And ‘ Where is your protein now?’
2. Why do we use lysozyme?
3. Why do we use Benzonase/Cyanase?
4. After each step ask – ‘Where is your protein now?’

Waster, Flow Through, Elution 1, Elution 2

1. What is different about the Wash vs. the Elution buffer?
2. Ask them to predict

How pure will your protein be?

Which sample has the most total protein?

BEFORE CLEANUP: - make sure they saved their **Sample 2, Sample 3, Sample 4, Sample 5, Sample 6**

**TROUBLESHOOTING:**

If there Elution 1 doesn’t come out purple – they should re-incubate their Flow Through and Wash samples together with the Ni-NTA for 15 min and then let it run through again – to a new **Elution 3 tube**

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